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Mosaic Rearrangement of Chromosome 18: Characterization by FISH Mapping and DNA Studies Shows Trisomy 18p and Monosomy 18p Both of Paternal Origin

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Structural abnormalities of chromosome 18p mainly consist of isochromosomes of the short arm, which result in tetrasomy 18p. Trisomy 18p is much rarer, and less well characterized. We report on a 12-year-old girl with minor facial anomalies, delayed development, abnormal hands, atopic dermatitis, and hearing loss. She was mosaic for two abnormal cell lines in peripheral blood. In 90% of cells, a dicentric chromosome with duplication of the whole short arm of chromosome 18 resulted in trisomy 18p; 10% of cells had monosomy 18p, arising from a t(14;18)(p11;q11). FISH mapping, with multiple region specific and locus specific probes from the short and long arm of chromosome 18, showed that the structure of the dicentric chromosome 18 was 18pter→18q23::18q11→18pter. DNA polymorphisms for chromosome 18 showed that the abnormalities of chromosome 18 were paternal in origin. Combining all results, we could link the trisomy 18p and monosomy 18p to a common origin via a complex series of events in an early mitosis. Am. J. Med. Genet. 92:101–106, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: mental retardation; dicentric chromosome; hearing defect

INTRODUCTION

Structural rearrangements involving chromosome 18p comprise translocations, deletions, and isochromosome formation. Isochromosome 18p appears to be the most common, and the resulting tetrasomy 18p is associated with a distinct syndrome [Callen et al., 1990]. Whole complete trisomy 18p is much less commonly reported. Most cases of trisomy 18p have resulted from inheritance of an unbalanced segregant from a balanced parental translocation. The phenotype in these cases was not only due to the trisomy 18p but also influenced by the partial monosomy in the derivative chromosome. Excluding translocations, the cytogenetic origin of other cases of isolated trisomy 18p, complete or partial, has been complex [Moog et al., 1994; Woolf et al., 1991]. From these few cases, the phenotype of trisomy 18p is variable, without a characteristic facial appearance and intellectual functioning normal or nearly normal [Moog et al., 1994; Takeda et al., 1989; Taylor et al., 1975; Wolff et al., 1991]. Whole arm monosomy 18p, derived from deletion or from translocation with an acrocentric short arm [Wang et al., 1997] shows a variable phenotype, usually including short stature, and mental and developmental delay [Jones, 1997].

Few cases of pure whole arm trisomy 18p or monosomy 18p have been studied at the molecular level. Using specific probes from chromosome 18, the origin of one case of pure trisomy 18p was a tandem duplication (18p) [Wolff et al., 1991] and an inverted duplication (18p) was shown in another [Moog et al., 1994]. We present a patient with duplication 18p together with monosomy 18p. The cytogenetic abnormality was characterized by FISH and DNA mapping with multiple probes from chromosome 18.

CLINICAL REPORT

The patient was born to healthy nonconsanguinous parents when both were 30 years of old. The family

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history showed epilepsy and intellectual impairment in both male and female relatives of the mother's family and the mother herself was mildly mentally retarded. The father, of normal intelligence, was adopted and had no information about his biological family. The patient had two older brothers, one of whom has an attention deficit disorder, and there were two early spontaneous pregnancy losses. The pregnancy was complicated by bleeding at 7 months. She was born at 42 weeks gestation by normal vaginal delivery with birth weight of 2.8 kg (10th centile). She required a humidicrib and assisted feeding for 8 days after birth. Subsequent psychomotor development was slow. Hearing loss was evident by age 2 years.

At 12 years the patient had severe intractable, atopic dermatitis, aggravated by self-excoriation. She had very small ear canals and moderate deafness with a loss of 30–50 db, requiring the use of a hearing aid. The deafness was a mixed sensorineural and conductive deafness. Speech development was markedly delayed. There was no history of epilepsy. On examination, weight was 47.8 kg (75th centile), height 141.2 cm (>3rd centile), head circumference 52 cm (10th centile), palm length 9 cm (<25th centile), middle finger length 5.5 cm (<3rd centile), and foot length 21.5 cm (10th centile). The palate was high but not narrow. The hands were abnormal with marked bilateral clinodactyly and camptodactyly of the 5th fingers, absence of distal creases on 4th fingers bilaterally, and the 2nd toe was longer than the 1st toe bilaterally. She was hyporeflexic at the knees and elbows. She had a thickened right nostril (Fig. 1). Thus, at 12 years she had normal facial appearance, short, abnormal fingers, deafness, obesity, relatively short stature, atopic dermatitis, and mental retardation.

On investigation, normal levels were obtained for serum lactate, serum pyruvate, IGF-1, growth hormone response to exercise, free thyroid hormone (T4) and (TSH), 24-hr urinary free cortisol, full blood count, creatine kinase, serum calcium, serum phosphate, lipid studies, and renal and liver biochemistry. She had normal cerebral and inner ear CT scans. The IgE level was >3,000 IU/ml on one occasion and >4,000 IU/ml on another occasion (normal range 0–120), demonstrating a marked hyper IgE state. Radiographs showed bone age delayed by 18 months.

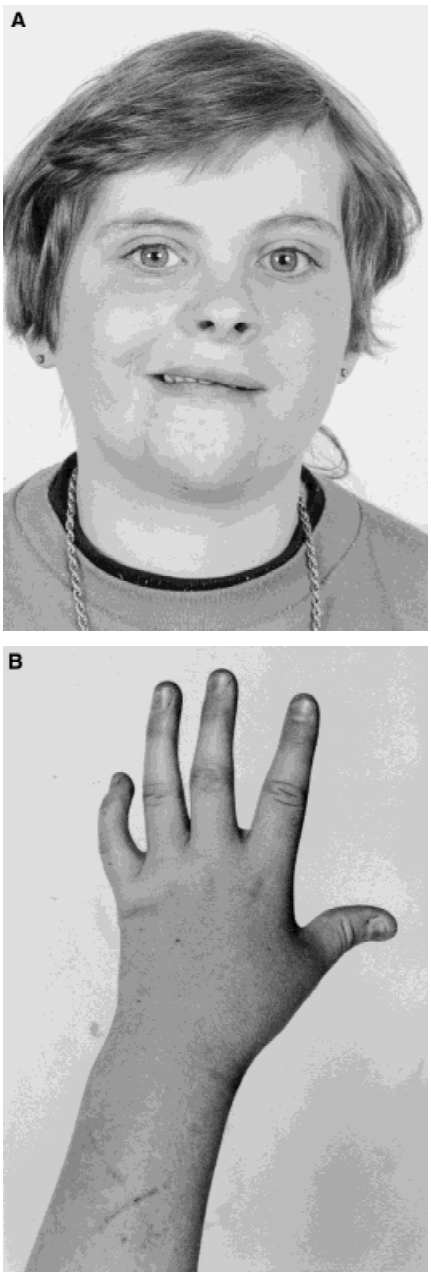

Fig. 1. The patient at 11 years of age; facial appearance **(A)** and left hand **(B)**.

METHODS AND RESULTS

Cytogenetics

Cytogenetics on peripheral blood was performed on referral and repeated 12 months later. Peripheral blood lymphocytes were studied after 72-hr PHA stimulated culture. Fragile X was negative. GTG banding revealed two cell lines—one contained an abnormal chromosome 18 and the other a translocation t(14;18). The abnormal chromosome 18 appeared to contain a normal short arm, centromere, and proximal long arm region with additional material of unknown origin on the distal long arm of chromosome 18 (Fig. 2A). Subsequent CBG banding showed the abnormal chromosome 18 to be dicentric, with only one primary constriction (not shown). Karyotype was 46,XX,dic(18). The frequency of the translocation cell line was 7 cells in 80 (8.75%) on the first test and 6 cells in 50 (12%) on the second test. The karyotype of this cell line was 45,XX,der(14)t(14;18)(p11;q11),–18 (Fig. 2B). Parental chromosomes were normal. Unfortunately, due to family difficulty with the many tests the patient required, the collection of additional samples (e.g., skin, cheek swabs, hair follicle cells) to determine body distribution and level of mosaicism was not possible.

FISH

Slides were made for FISH analysis from the suspension remaining after the cytogenetic harvest. The

Fig. 2. Partial GTG banded karyotypes. On the left side of the bar is the cell line with t(14;18)—the normal 14 on the left, the der(14) in the center, and the normal chromosome 18 on the right. On the right side of the bar is the cell line with the dic(18) (right) and the normal chromosome 18 (left).

whole chromosome paint for 18 (CHR18B, CAMBIO, Cambridge, UK) fully painted the normal chromosome 18 and the dic(18) as well as one arm of the der(14) (not shown). A chromosome 18 α satellite probe (for the centromeric locus D18Z1, Oncor, Gaithersburg, MD) confirmed that the abnormal chromosome 18 was dicentric (Fig. 3a). The t(14;18) chromosome did not give a signal with the 18 centromere probe (Fig. 3b).

Subsequent hybridization with a microdissection probe specific for 18p [Guan et al., 1996] showed that the additional material was of 18p origin (Fig. 4a). A further six probes from specific regions of chromosome 18 were used, in separate and combined hybridization procedures: microdissection probes for 18p and 18q arms, telomeric YAC clones for 18p11.3 (TYAC89) and 18q23 (TYAC45) [Vocero-Akbani et al., 1997], and locus specific probes for 18q12 (PAC 237H11) and 18q21 (YAC ICRFy900B1016), the latter two probes proving that the abnormal 18 was not an isochromosome.

The dic(18) contained 18p telomeres at both ends (Fig. 4a) and did not contain the 18q telomere at all (Fig. 4d). The der(14) contained the 18q telomere (Fig. 4e). Both 18q12 and 18q21 probes gave one positive signal in the long arm of the dic(18) (Fig. 4b and c, respectively). The signal obtained with the PAC 237H11 probe placed the breakpoint more distal than 18(q12). The additional 18 long arm probe (YAC ICRFy900B1016) was also present as a single band, thus placing the breakpoint distal to 18(q21). Matching the FISH breakpoints with GTG bands, bands q22 and 23 were intact, localizing the lower breakpoint on chromosome 18 to q23—just proximal to the telomere. For the abnormal chromosome 18 to be derived from a normal chromosome 18, two breaks were required—one proximally on the long arm, just under the centromere (q11) and the other distally at 18q23, with the 18p segment (containing the centromere) attaching to the 18q23 region. The structure of the dic(18) was 18pter→18q23::18q11→18pter. Summarizing the number of cells examined with the various FISH techniques as well as the GTG and C banding gave an overall frequency of 10% for the monosomic 18p cell line. The full karyotype, based on ISCN [1995], is 46,XX,dic(18)(q23q11)[17]/45,XX,der(14)t(14;18)(p11;q11),–18[13].

DNA Studies

The following microsatellite markers were typed using genomic DNA of the patient and her parents: 18pter-D18S9-PACAP-D18S453-D18S40-cen-D18S46-MBP-D18S497-18qter. The definition of loci on chromosome 18 was based on gene mapping information [Genome Database, 1996]. The maximal distance between the most proximal marker in 18p, D18S40, and the centromere is 3.7 cM [Eggermann et al., 1996]. By analysis of 18p-specific microsatellites, we were able to demonstrate that the chromosomes 18 involved in the rearrangement were of paternal origin (Table I). In all five analyzed markers, the paternally inherited allele showed double the intensity of the maternally inherited allele (Table I). Furthermore, in each marker paternal heterozygosity was reduced to homozygosity in the child. Typing of long arm markers indicated a regular biparental inheritance. Based on the observed homozygosity of all markers spanning the whole short arm of chromosome 18, we concluded that there was a postzygotic error, with involvement of the paternal alleles. Paternal UPD for 18p could be excluded as maternal alleles (D18S59, D18S452, and D18S453) were demonstrated. The rate of 10% monosomic 18p cells was too low to alter the differentiation between the PCR amplified alleles.

Proposed mechanism of the trisomy 18p/monosomy 18p cell lines. In the interpretation of our patient's karyotype, the common breakpoint on 18q11 relates the t(14;18) and the dic(18) abnormalities. A mitotic error involving the paternal chromosome 18 occurred in a normal zygote at the S phase or G2 in a very early mitosis, possibly the first. There was a break in one chromatid at 18q11 and in the other at 18q23 (Fig. 5). There was also a break in a 14p11 chromatid. The long arm of one chromosome 18, distal to the break in 18q11, translocated onto chromosome 14 giving rise to the der(14) (which on FISH did not contain the 18 centromere). At the same time, the 18 short arm segment containing the centromere became attached to the 18q23 of the other chromatid. The very terminal segment of this 18 long arm was subsequently lost as was the 14 short arm fragment. Upon segregation at metaphase, one cell line was monosomy 18p and the other trisomy 18p. This hypothesis accounts for the three breaks that must have occurred and fits in with all observed cytogenetic, FISH, and DNA findings.

An alternative mechanism that the patient was a trisomy 18 zygote, with two copies of the paternal 18 (which subsequently had a break in each), arising by M2 nondisjunction or postzygotic nondisjunction, is not tenable. The homozygous state for 18p markers in the child makes a meiotic error leading to trisomy 18 virtually impossible (The most proximal marker in 18p has a maximum distance to the centromere of 3.7 cM.). Additionally, there was no hint on the microsatellite gels for a mosaicism in respect to a third allele which allows the delineation of a meiotic nondisjunction.

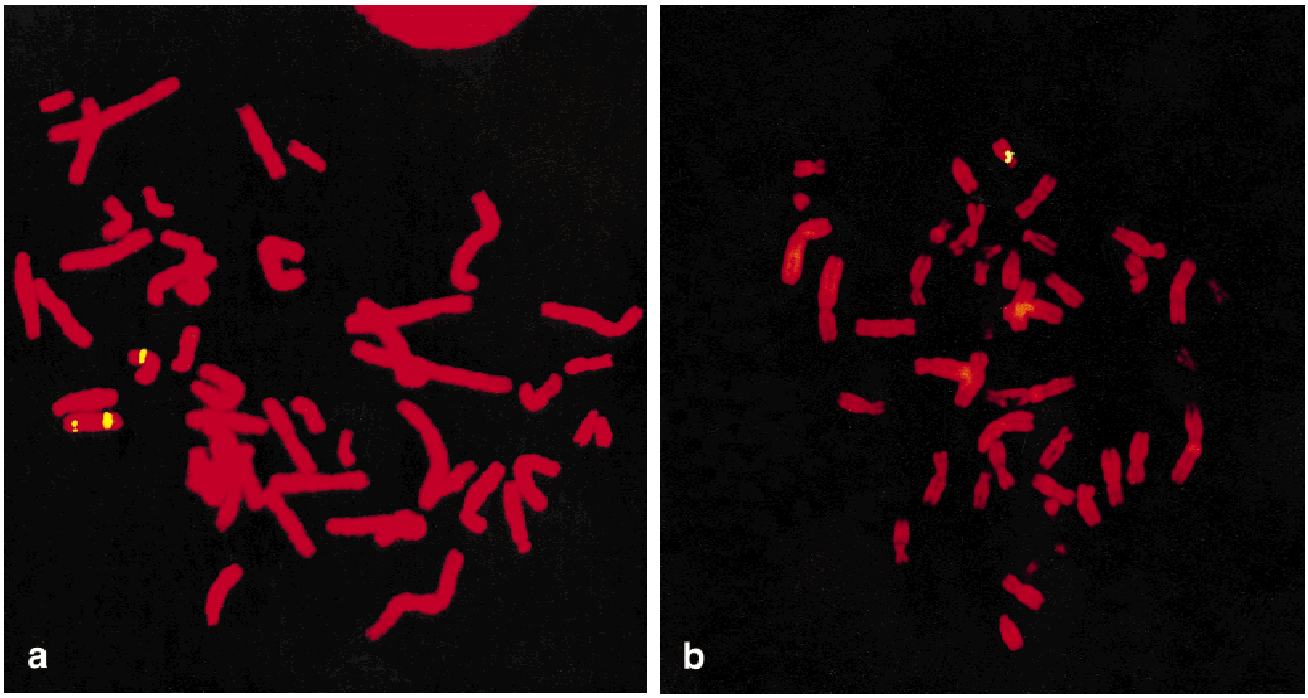

Fig. 3. Metaphase spread with FISH and the 18 centromere probe from the cell line with the dic(18) (2 signals; normal 18 one signal) **(a)**; 18 centromere probe (LI.84- FITC) in the cell line with the t(14;18) **(b)**. Only one signal is present, on the normal chromosome 18, proving that the t(14;18) is a derivative chromosome 14.

DISCUSSION

The rearrangement described here is unique and is the only report of an additional 18p attached to the 18 long arm. We could find only seven previous cases (in four reports) of pure whole short arm trisomy 18 and in these, the structure of the abnormal chromosome 18 varied. In two, ascertainment was through a child with tetrasomy 18p born to a mother with trisomy 18p [Takeda et al., 1989; Taylor et al., 1975]. No DNA work

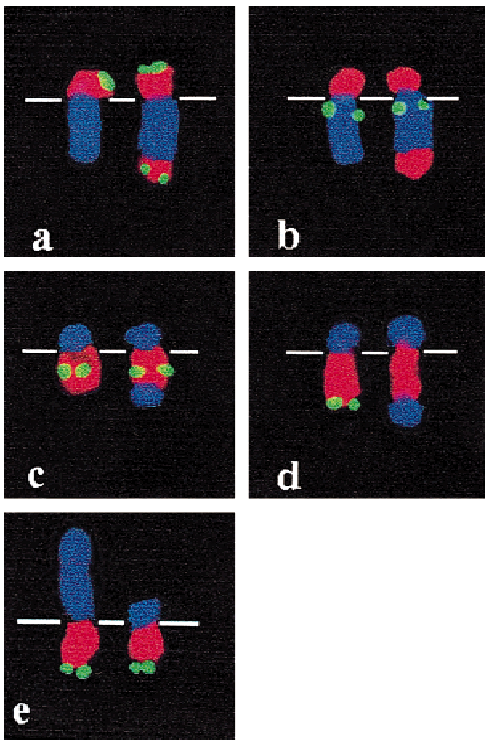

Fig. 4. Digitized pseudo-colored images of the normal chromosome 18 on the **left** and dic(18) or t(14;18) on the **right** from five separate dual-color hybridizations. **a:** Hybridization of the microdissection probe for 18p arm detected in red together with a telomeric YAC clone for 18p11.3 (TYAC89) detected in green fluorescence. **b:** Microdissection probe for 18p arm (red) hybridized together with a PAC clone from 18q12 (237H11) (green). **c:** Hybridization of the microdissection probe for 18q arm detected in red together with a YAC clone for 18q21 (ICRFY900B1016) in green. **d:** Microdissection probe for 18q arm (red) hybridized together with a telomeric YAC clone for 18q23 (TYAC45)(green). **e:** Same hybridization mixture as **d** for metaphase chromosomes carrying the translocation chromosome t(14;18).

TABLE I. STR Typing Results for 18p Markers*

STR locus	Father		Mother		Patient		Informativity	18p
D18S59	3	4	1	2	1	4[a]	Paternal	R[b]
PACAP	1	3	2	3	1[a]	3	Paternal	R
D18S452	1	3	2	4	2	3[a]	Paternal	R
D18S453	1	2	3	3	2[a]	3	Paternal	R
D18S40	2	3	1	4	1	3[a]	Paternal	R

*STR typing results for 18q markers not shown.
[a]Alleles showing stronger intensities.
[b]R = reduction of paternal heterozygosity.

had been performed in these cases. In two other patients with trisomy 18p, DNA studies showed tandem dup(18p) [Wolff et al., 1991]. In a further three patients, FISH showed a direct tandem duplication of 18p in two and one had an inverted duplication of 18p [Moog et al., 1994]. Although the trisomy of 18p due to the direct duplication had resulted in a dicentric chromosome 18 (as in our case), this dicentric was missing the terminal 18p band [Moog et al., 1994]. The inversion duplication was also deleted for the terminal 18p band, and FISH confirmed the location of the 18 centromere to be terminal [Moog et al., 1994]. In our dicentric chromosome, there is no 18p material missing, as confirmed by the FISH localization of the 18p telomere YAC clone. Finally, in one other report comprising a mother and child [Wolff et al., 1991], the duplicated 18p chromosome was not dicentric, again different from the duplicated 18p described here.

The phenotype of our patient has putative contributions from trisomy 18p, monosomy 18p, and the small terminal 18q deletion. As our patient is mosaic for a cell line with trisomy 18p (majority) and one with monosomy 18p (minority) in peripheral blood, it is not possible to be certain of the phenotypic impact made by either one alone, as monosomy may contribute more to phenotypic abnormality than trisomy for the same segment [Schinzel, 1993]. Also, their frequency in other tissues is unknown. Peripheral blood was the only tissue examined in all reported cases, and some comparisons with other apparently nonmosaic cases of trisomy 18p can be made. In the four reports of seven patients with pure whole arm trisomy 18p, the 5 female patients of reproductive age were not infertile [Moog et al., 1994; Takeda et al., 1989; Taylor et al., 1975; Wolff et al., 1991], mental development was normal in four, there were no specific minor facial anomalies, and microcephaly, abnormal hands with bilateral short 5th fingers, growth delay, and poor speech were the main manifestations. Our patient has the latter three findings but appears to be more severely affected. Deafness could contribute to the delayed mental development in our patient. Deafness is a known finding in the 18q- syndrome, located very terminally on 18q [Bogosian-Sell et al., 1994], and our patient had the very small ear canals seen in other patients with 18q-. Abnormal somatic growth, mental retardation, and immunological and limb anomalies are also part of deletion 18q- [Kline, 1993], thus we cannot exclude an effect from the undetectable, minimal subtelomeric deletion of 18q in the dic(18) chromosome (18q23→qter) contributing to the phenotype in our patient.

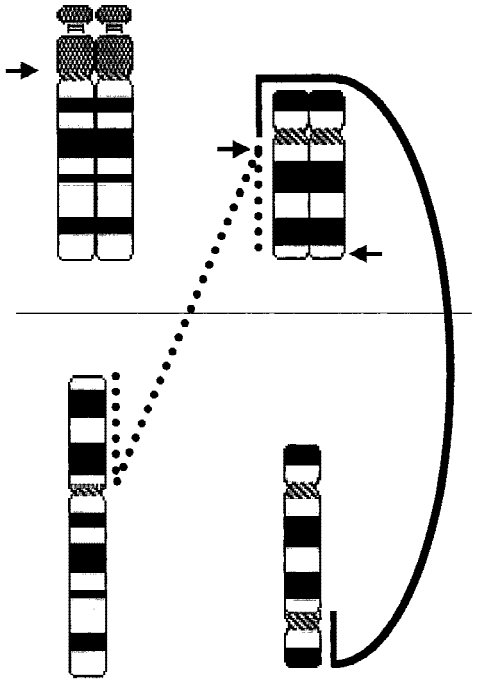

Fig. 5. Mechanism of formation of the complex karyotype shown in ideograms. Above the horizontal line are the paternal chromatids—the 14 on the **left** and the 18 on the **right**. Arrows indicate the breakpoints in the chromatids. Below the horizontal line are the resulting abnormal chromosomes—one being dicentric with 18p attached to the 18q and the other with 18q attached to 14p. There is no normal paternal chromosome 18.

The complex events producing the abnormal chromosomes 18 described here occurred in the paternally derived chromosome 18. Trisomy 18 has been shown to be paternal in up to 10% of cases [Eggermann et al., 1996; Fisher et al., 1995; Yagang et al., 1993] but thus far, 6 of the 8 cases of paternal trisomy 18 documented were the result of mitotic nondisjunction. The marker typing results of our patient support the observation that segregation errors of paternal chromosomes 18 in meiosis are a rare event. Another case of a rare dicentric chromosome 18 was also paternal in origin [Gravholt et al., 1997]—it may be that structural rearrangements of chromosome 18 occurring postzygotically preferentially involve the chromosome 18 from the father.

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